

- 18 -

What is claimed is:

1. An isolated liver cell cluster comprising a liver stem cell and a hepatocyte.
2. The liver cell cluster of claim 1, wherein 5 said liver cell cluster is a cell doublet.
3. The liver cell cluster of claim 1, wherein said hepatocyte and said stem cell are joined by a desmosomal junction.
4. The liver cell cluster of claim 1, wherein 10 said stem cell is a pre-oval cell.
5. The liver cell cluster of claim 1, wherein said stem cell expresses OV6.
6. The liver cell cluster of claim 1, wherein said stem cell expresses a bile duct cell marker.
- 15 7. The liver cell cluster of claim 6, wherein said bile ductal cell marker is a cytokeratin.
8. The liver cell cluster of claim 7, wherein said cytokeratin is cytokeratin 19.
9. The liver cell cluster of claim 3, wherein 20 said stem cell expresses desmoplakin.

- 19 -

10. The liver cell cluster of claim 6, wherein  
said stem cell is further characterized as expressing an  
antigen selected from the group consisting of laminin,  
desmoplakin I, cell-cell adhesion molecule (CCAM),  
5 carcinoembryonic antigen (CEA), dipeptidyl peptidase-4,  
 $\gamma$ -glutamyl transpeptidase ( $\gamma$ GT), Very Late After  
Activation (VLA)-2, VLA-3, VLA-5, and VLA-6.

11. The liver cell cluster of claim 1, wherein  
said cluster is derived from adult liver tissue.

10 12. The cluster of claim 1, wherein said cluster  
is derived from a fetal or pediatric liver.

13. The liver cell cluster of claim 1, wherein  
said cluster is derived from human tissue.

14. The liver cell cluster of claim 1, wherein  
15 said cluster is derived from rodent tissue.

15. The liver cell cluster of claim 1, wherein  
said stem cell differentiates into a mature functional  
hepatocyte or a bile duct cell.

16. The liver cell cluster of claim 1, wherein  
20 said stem cell comprises heterologous DNA encoding a  
therapeutic protein.

- 20 -

17. The liver cell cluster of claim 16, wherein said therapeutic protein is selected from the group consisting of ornithine transcarbamylase, arginosuccinate synthetase, glutamine synthetase, glycogen synthetase, 5 glucose-6-phosphatase, succinate dehydrogenase, glucokinase, pyruvate kinase, acetyl CoA carboxylase, fatty acid synthetase, alanine aminotransferase, glutamate dehydrogenase, ferritin, low density lipoprotein (LDL) receptor, alcohol dehydrogenase, 10 albumin, transferrin, complement component C3,  $\alpha_2$ -macroglobulin, fibrinogen, Factor XIII:C, Factor IX, or  $\alpha_1$ -antitrypsin.

18. A primary liver stem cell, wherein said stem cell is

15 (a) obtained from normal liver tissue, and  
(b) derived from an isolated liver cell cluster comprising a hepatocyte and said stem cell.

19. The stem cell of claim 18, wherein said liver cell cluster is a cell doublet.

20. The stem cell of claim 18, wherein said hepatocyte and said stem cell are joined by a desmosomal junction.

21. The stem cell of claim 18; wherein said stem cell is a pre-oval cell.

25 22. The stem cell of claim 18, wherein said stem cell expresses OV6.

23. The stem cell of claim 18, wherein said stem cell expresses a bile duct cell marker.

- 21 -

24. The stem cell of claim 23, wherein said bile ductal cell marker is a cytokeratin.

25. The stem cell of claim 24, wherein said cytokeratin is cytokeratin 19.

5 26. The stem cell of claim 20, wherein said stem cell expresses desmoplakin.

27. The stem cell of claim 23, wherein said cell is further characterized as expressing an antigen selected from the group consisting of laminin, 10 desmoplakin I, CCAM, CEA, dipeptidyl peptidase-4,  $\gamma$ GT, VLA-2, VLA-3, VLA-5, and VLA-6.

28. The stem cell of claim 18, wherein said stem cell is derived from adult liver tissue.

29. The stem cell of claim 18, wherein said stem 15 cell is derived from a fetal or pediatric liver.

30. The stem cell of claim 18, wherein said stem cell is derived from human tissue.

31. The stem cell of claim 18, wherein said stem cell is derived from rodent tissue.

20 32. The stem cell of claim 18 wherein said stem cell differentiates into a mature functional hepatocyte or a bile duct cell.

33. The stem cell of claim 18, wherein said stem cell comprises heterologous DNA encoding a therapeutic 25 protein.

- 22 -

34. The stem cell of claim 33, wherein said therapeutic protein is selected from the group consisting of ornithine transcarbamylase, arginosuccinate synthetase, glutamine synthetase, glycogen synthetase, 5 glucose-6-phosphatase, succinate dehydrogenase, glucokinase, pyruvate kinase, acetyl CoA carboxylase, fatty acid synthetase, alanine aminotransferase, glutamate dehydrogenase, ferritin, LDL receptor, alcohol dehydrogenase, albumin, transferrin, complement component 10 C3,  $\alpha_2$ -macroglobulin, fibrinogen, Factor XIII:C, Factor IX, or  $\alpha_1$ -antitrypsin.

*Seal* 35. A method of obtaining a sample of isolated liver stem cells comprising

15 (a) isolating a liver cell cluster from normal liver tissue, said cluster comprising a stem cell associated with a hepatocyte;  
20 (b) dissociating said stem cell from said hepatocyte; and  
(c) removing said hepatocyte from said doublet to yield a sample of liver stem cells.

36. The method of claim 35, comprising the step of enriching for periportal hepatocytes associated with the biliary tree.

37. The method of claim 35, wherein said liver 25 cell cluster is a cell doublet.

38. The method of claim 35, wherein said liver cell cluster is derived from the canal of Hering of an adult liver.

- 23 -

39. The method of claim 35, further comprising selecting for expression of desmoplakin.

40. The method of claim 35, further comprising selecting for expression of OV6.

5 41. The method of claim 35, further comprising selecting for a cell which expresses an antigen selected from the group consisting of laminin, desmoplakin I, CCAM, CEA, dipeptidyl peptidase-4,  $\gamma$ GT, VLA-2, VLA-3, VLA-5, and VLA-6.

10 42. A liver stem cell isolated according to the method of claim 35.

43. An extracorporeal liver assist device comprising the liver cell cluster of claim 1.

15 44. An extracorporeal liver assist device comprising the liver stem cell of claim 18.

45. A method of hepatic transplantation, comprising transplanting into a mammal the liver cell cluster of claim 1.

20 46. A method of hepatic transplantation, comprising transplanting into a mammal the stem cell of claim 18.

25 47. A method of treating an inherited or acquired genetic or metabolic disease in a mammal comprising transplanting into said mammal the liver cell cluster of claim 16.

- 24 -

48. A method of treating an inherited or acquired genetic or metabolic disease in a mammal comprising transplanting into said mammal the stem cell of claim 33.

Claim 2

Applicant's Remarks